

THE LIFE HISTORY OF THE TREMATODE *ALARIA MUSTELAE*,
SP. NOV.

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY, IN THE UNIVERSITY OF
MICHIGAN

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Reprinted from TRANSACTIONS OF THE MICROSCOPICAL SOCIETY
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THE LIFE HISTORY OF THE TREMATODE
ALARIA MUSTELAE, BOSMA, 1931^{1,2}

NELLY J. BOSMA

INTRODUCTION

Alaria mustelae Bosma has been briefly diagnosed and the outlines of its life history related in a preliminary paper (Bosma, 1931). Of the number of studies made on the Strigeoidea, notably those of Lutz (1921, 1929), Ruskowski (1922), Szidat (1924a, 1924b), Mathias (1925), Faust (1927), and Van Haitsma (1930, 1931a, & 1931b), none pertaining to a member of the genus *Alaria*, the oldest genus in the superfamily Strigeoidea, has been completed.

The life cycle of *Alaria mustelae* presents a new feature in that the larval period between the cercaria and mature metacercaria is considerably prolonged, and two hosts are required for the metamorphosis from cercaria to metacercaria instead of one. A prolongation, and an arrest in the development, of the early metacercarial stage occurs in the second intermediate host, while the regular metacercarial phase which has precocious adult tendencies occurs in the third intermediate host. This early metacercarial stage is similar to the larval form described by La Rue (1917) as *Cercaria marcianae* and which Cort (1918) called *Agamodistomum marcianae*. There are therefore the following hosts and stages in the development of *Alaria mustelae*: a snail which harbors the sporocysts producing cercariae, a tadpole or frog into which the cercariae penetrate and in which they become immature metacercariae or agamodistomes, here to be called *mesocercariae* (defined below), a mammal (mouse, mink, or raccoon) which carries the metacercariae, and another mammal (mink or weasel) the host of the hermaphroditic adult. Weasels and minks both belong to the genus *Mustela* which suggested *Alaria mustelae* as the name for the species.

I wish to express my sincere appreciation to Prof. George R. LaRue, under whom this work was conducted, for many helpful suggestions and for the interest shown in its progress. I also wish to thank Dr. Helen F. Price who gave me specimens of adult worms from a weasel, Mr. Henry Vander Schalie who identified the snail host of the cercaria, Mr. Donald Ameel who gave me information concerning the eating of minks by dogs, and all friends and associates who have aided in the collection of material.

MATERIALS AND METHODS

Many experimental animals were employed including snails, tadpoles and frogs, mice, rats, minks, weasels, ferrets, raccoons, cats and dogs, some

¹ Contribution from the Department of Zoology, University of Michigan. This is number 27 in the series of studies on the trematode family Strigeidae and the superfamily Strigeoidea by Dr. G. R. La Rue, his students and associates.

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of which had been naturally infected with certain stages of the parasite while the others were used for infection experiments. The snail host is *Planorbula armigera* (Say) which was collected from a shallow pool about three miles southeast of Ann Arbor, Michigan, during the spring seasons of 1929 and 1930. Not many snails of this species were found and therefore no estimate of the percentage of infections with this cercaria could be made. The snails were placed in shell vials in tap water and observed daily for the emergence of cercariae. Living cercariae were used for infection experiments on laboratory animals. For study, mounts were made of living cercariae both unstained and stained with various *intra vitam* dyes of which neutral red, Nile blue sulphate, brilliant cresyl blue, toluidine blue, and Janus green B were found useful to bring out certain structures. The cercariae were fixed in hot 10% formalin for measuring.

Tadpoles from the same pool from which the snails were collected yielded mesocercariae which were used for study, while tadpoles of *Rana pipiens* and *R. clamitans* used for infection experiments came from a pool northwest of Ann Arbor in which no snails of the species *Planorbula armigera* could be found. To make certain that they were uninfected the tissues of several of them were carefully examined for larval trematodes. Frogs collected from various places in the neighborhood of Ann Arbor were examined for mesocercariae and it was found that *Rana clamitans* and *R. catesbeiana* were more often and more heavily infected than *R. pipiens* and *R. palustris*, although occasionally a heavy infestation might be found in either of the latter. One frog, *R. catesbeiana*, with a particularly heavy infestation of which more will be said later, came from Third Sister Lake, three miles west of Ann Arbor. Many mesocercariae were studied alive, particularly for the excretory system. Others were fixed in Gilson's solution, stained in paracarmin and mounted *in toto*. These were used for measurements.

Laboratory raised rats and mice, which received only a balanced grain ration, dog biscuit and lettuce, were used as hosts for certain feeding experiments. The muscles of two mice, *Peromyscus leucopus noveboracensis*, collected near a pool west of Ann Arbor yielded small white cysts containing metacercariae which were used for comparative study with metacercariae from other sources.

Another investigation being carried on in this laboratory made available for examination the carcasses of many minks and some raccoons during the fall and early winter of 1930. The localities from which these carcasses came were quite generally distributed throughout the whole lower peninsula of Michigan, but those having the greatest number of metacercariae usually came from the southern half of the state. The first minks were examined as the mice had been, that is, the muscles of the legs and throat, the intercostals and the diaphragm were carefully searched under a magnifying glass for small white cysts, and any small suspicious appearing mass was exam-

ined under the compound microscope. Metacercariae were found in all these muscles, but the diaphragm was most heavily infected. However, many more were found in the lungs than in any other part of the body. A smaller number of raccoons than minks were examined and a smaller percentage of raccoon lungs were infected, and these less heavily than the lungs of the mink.

The metacercariae were easily freed by tearing the lung tissue with needles. Since this process proved to be very time consuming, a more expeditious method was sought. After removal of the largest bronchi the lung was very finely chopped up by cutting with scissors after the method used for examination of muscle for *Trichinella*. Salt solution was poured on the mass which was then stirred, and allowed to settle. Much of the lung tissue floated to the top and was poured off. If there was much blood in the lungs it was necessary to add fresh salt solution and repeat the settling and decanting process until the solution was fairly clear. This solution with sediment was put in a shallow glass dish and examined under the binocular dissecting microscope. This method failed to secure all the metacercariae present in a lung, but the large numbers of larvae usually present made this loss of little consequence. Living mesocercariae and specimens prepared as whole mounts were compared with those obtained from frogs. The developmental series of the metacercariae was studied alive for the excretory system and other organs. No advantage was found in the use of *intra vitam* stains on the metacercariae. Metacercariae were fixed in Gilson's solution and stained in paracarmin for whole mounts, and others were used for transverse or sagittal sections. Sections were stained in Mayer's acid haemalum and erythrosin.

Two dogs which were also being used in feeding experiments of *Dipyllobothrium latum* were used as final hosts. To the first dog the lungs of one mink, after removal of a small portion of the lung for examination to make certain that metacercariae were present, were given by forced feeding. The other dog received a piece of meat in which many freed metacercariae had been embedded, as well as a lung containing many larvae. About two weeks later fecal examination showed the presence of eggs, after which the dog was killed; the intestine removed, slit open, shaken and scraped in salt solution. Mucus and other flocculent material floated to the surface and was poured off while the heavier material and worms settled to the bottom. By repeating this process several times the solution became clear and the trematodes were easily picked out.

A cat was also used as final host. Fecal examination revealed no parasite eggs. This cat by forced feeding was given the lungs of a mink. Further handling was like that given the dogs. Other hosts were two ferrets secured from a breeder in Ohio. Neither showed any parasite eggs when fecal examination was made. One ferret was given metacercariae removed from

mink lung and placed in meat. The other was fed mice which had previously been given mesocercariae. After finding eggs in the feces which gave assurance that the worms were developing in the intestines time was given so that all the worms might come to maturity before the animal was killed. Examination for the worms was conducted as with the dogs and cat.

The intestines of a few minks and also of a weasel were examined for parasites, and adult trematodes of the genus *Alaria* were found.

The worms were anaesthetized with chloretone, and then fixed in Gilson's solution. Some were stained in paracarmine or alcoholic cochineal for whole mounts, and a few others were sectioned, either transversely or sagittally. Sections were stained with acid haemalum and erythrosin. Reconstructions of the various systems were made from drawings made by means of the camera lucida or the microprojection apparatus.

LIFE HISTORY

For some time it had been my wish to study the life-history of *Agamodistomum marcianae* (La Rue), one of the strigeids whose cercaria much resembles *C. douglasi* Cort (1917). The latter upon penetration into snails produces a tetracotyle while the former, as shown by Cort and Brooks (1928), upon entrance into a tadpole becomes one of those intermediate, postcercaria-like forms known as agamodistomum. When the tadpoles containing these agamodistomes were fed to garter snakes the parasites migrated into the tissue of the snake but remained unchanged. This phenomenon has been one of the most inexplicable peculiarities in life history known to parasitologists. Therefore, the determination of the true fate of the cercaria and agamodistomum would be a most interesting problem the fundamental question of which would be: is this *Agamodistomum marcianae* only an aberrant form and the tadpole a blind alley, or is it an essential step in a life cycle, and what is the nature of the adult? Lutz (1929) fed agamodistomes from *Hyla crepitans* to a dove and young duck and recovered tetracotyles from them after a period of 6 and 12 days respectively but gave no description of either form. He concluded that this species required four hosts. This paper was unknown to me until after my work had been completed.

During the spring of 1929 a few small snails, *Planorbula armigera*, were brought into the laboratory, from one of which tiny fork-tailed cercariae were emerging. At first these were considered to be *C. marcianae*. Detailed examination of the cercaria showed that the characters given by Cort and Brooks (1928) did not fit in all particulars. When a tadpole was exposed to the cercariae from *Planorbula armigera* it was immediately attacked on all sides, and in a few minutes almost all the cercariae had penetrated. Several parasite-free tadpoles exposed to these cercariae, and examined after two weeks contained forms resembling *Agamodistomum marcianae*, but a close

comparison again revealed significant differences in a sufficient number of characters to make the two forms distinguishable.

At about this time a large bullfrog, *Rana catesbeiana* was found heavily parasitized with very small cysts, most of which were transparent, and the remainder gray or black. They were packed between the muscle fibers, between muscles, in the lymph spaces, and in the wall of the alimentary canal. There were living agamodistomes in the transparent cysts and dead atrophying worms in the black cysts. When these agamodistomes were carefully studied and compared with *A. marciana*, and with those grown in the experimentally infected tadpoles, they were considered to be the same as the latter. Usually in the tadpoles no cysts were formed and in the frogs some of the parasites were without cysts, but structurally no difference could be detected in the parasites.

Three laboratory-raised white rats were given pieces of the muscle of the bull-frog containing the cysts. Examination of one rat two weeks after the feeding produced no results. This result has since been explained on the ground that the parasites at this stage are so small that they are overlooked. The next rat was examined seven weeks after the feeding and twelve cysts were found in the muscles, between the fibers. Unfortunately the lungs of these animals were not examined, since it was not appreciated until later that these parasites collect in the lungs in greater numbers than elsewhere. When opened, metacercariae emerged from the cysts. These metacercariae were not considered to be full grown, however, because remnants of the penetration glands were still evident. One and a half weeks later the other rat was examined and this yielded seventeen cysts. The metacercariae now showed the ducts only in the oral sucker. These experiments demonstrated that the so-called agamodistomum of this species is an essential step in the development of the metacercaria and because of this fact it is given a distinctive name. Therefore, the name *mesocercaria* is suggested as appropriate for it.

During the autumn of 1929 several carcasses of small mammals were examined, including *Sorex cinereus cinereus*, *Blarina brevicauda talpoides*, *Microtus pennsylvanicus pennsylvanicus*, and *Peromyscus leucopus noveboracensis*. Two of the last yielded cysts containing metacercaria. That they were like those grown in the rat was determined by careful comparison of the structures, particularly the excretory bladder, and the measurements of the two forms. Measurements of these forms are included in Table I.

During the following spring (1930) more infected snails, *Planorbula armigera* (Say), were found, the experiments were resumed and several tadpoles were infected. It was now known that mice and rats could serve as hosts of the metacercariae and it had been established that rats had been parasitized by eating frog's flesh containing mesocercariae, so it was now desired to ascertain whether the mammalian host might not be parasitized

by penetrating cercariae. In order to test this matter, a mouse was placed in a dish having a small amount of water containing many cercariae. The water had been warmed to 33° C. because Dr. Helen Price of this laboratory had found that by raising the temperature of the water containing cercariae of *Schistosomatium douthitti* they became more active and penetrated into

TABLE I
MEASUREMENTS OF METACERCARIAE IN MILLIMETERS

Host	Rat			Mouse			Mink		
No. of specimens measured	7			6			10		
	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.
Total length	0.477	0.639	0.542	0.472	0.654	0.584	0.492	0.716	0.613
Hindbody, length	0.118	0.151	0.135	0.114	0.159	0.148	0.116	0.17	0.153
Hindbody, width	0.131	0.168	0.148	0.134	0.17	0.148	0.134	0.196	0.168
Oral sucker, length	0.06	0.075	0.067	0.062	0.075	0.07	0.062	0.089	0.075
Oral sucker, width	0.056	0.063	0.06	0.06	0.069	0.064	0.062	0.071	0.068
Pharynx, length	0.054	0.069	0.059	0.062	0.071	0.065	0.062	0.089	0.07
Pharynx, width	0.04	0.051	0.046	0.04	0.053	0.048	0.044	0.062	0.053
Acetabulum, length	0.051	0.059	0.053	0.053	0.06	0.058	0.053	0.062	0.06
Acetabulum, width	0.052	0.063	0.057	0.06	0.062	0.068	0.062	0.071	0.065
Holdfast, length	0.109	0.154	0.127	0.124	0.174	0.145	0.134	0.196	0.163
Holdfast, width	0.064	0.085	0.078	0.07	0.104	0.089	0.08	0.116	0.102

mice and rats more readily. The mouse appeared to be very uncomfortable, raising its fore-feet, rubbing them against each other, licking them and other wet parts of the body with its tongue, but whether this was because of discomfort produced by the entrance of the cercariae or only from the water could not be determined. The mouse was left in the water 20 minutes, and the next day the process was repeated. After removing the mouse from the water the cercariae seemed to be as numerous as at the beginning of the experiment, nor were there many unattached tails for which a body could not be found in the bottom of the dish.

Three other mice were given treatment similar to the first, except that for two the water was left at room temperature. Eight weeks after the exposure none of these yielded metacercariae, proving that mammals cannot be infected directly by these cercariae, and confirming the previous experiments which showed the mesocercariae in the tadpole or frog to be an indispensable step in the development of the metacercariae.

The first mink and raccoon carcasses were examined during the early fall of 1930. As these two animals are known to eat frogs the examination was very thorough, and metacercariae were found, not only in the muscle but also in the lungs, however in fewer numbers in the raccoon than in the mink. This was the first time that lungs had been given much attention and they proved to have greater numbers of metacercariae than any other part of the body. In them were found metacercariae in all stages of develop-

ment making it possible to study a series of developmental stages and thus to work out the development of the excretory and reproductive systems. The first carcasses examined had many very young worms in the lungs, apparently mesocercariae which had just entered. These mesocercariae aided greatly in arriving at the decision that these worms in the lungs of the mink were conspecific with those dealt with previously, for the small mesocercariae from the mink were similar to those from the tadpole in size (see table II), and in the possession of the same number and arrangement

TABLE II
MEASUREMENTS OF MESOCERCARIAE IN MILLIMETERS

No. of specimens	From frog muscle					
	10			10		
	Min.	Max.	Av.	Min.	Max.	Av.
Body, length	0.241	0.34	0.292	0.25	0.313	0.278
Body, width	0.143	0.205	0.179	0.143	0.196	0.17
Oral sucker, length	0.052	0.08	0.066	0.064	0.074	0.07
Oral sucker, width	0.042	0.07	0.059	0.046	0.06	0.053
Acetabulum, length	0.04	0.052	0.046	0.042	0.05	0.046
Acetabulum, width	0.048	0.062	0.053	0.048	0.066	0.052

of penetration glands, in the spination of the anterior part of the body and of the ventral sucker. Von Linstow (1875) had pointed out the similarity between *Agamodistomum tetracystis* from the frog and *A. putorii* from *Foetorius putorius*. The findings on the life history of the present species demonstrate the value of his judgment as it seems likely that these two will prove to be parts of the same life history.

The intestines of these minks contained adult strigeids belonging to the genus *Alaria*, as did also the intestines of a weasel, *Mustela noveboracensis noveboracensis*. Three similar worms found in the intestines of a weasel during the summer of 1929 were given to me by Dr. H. F. Price.

The question of the final host had now to be met. It might be either a mouse-eating bird or mammal. As a starting point a heavily infected mink lung was fed by force to a dog. Eleven days after the feeding a fecal examination showed strigeid eggs. Two days later the dog was killed and in the anterior third of the intestine twenty-six adult worms were found. They plainly belonged to the genus *Alaria* and even by superficial examination the resemblance to those from the intestines of the mink was noticeable.

Another dog was fed a more heavily infected lung and a large number of free metacercariae buried in a piece of meat. Eggs were found in the feces ten days after feeding, but because the worms from the first dog had had so few eggs in the uteri it was thought that they might not have reached full maturity, and therefore this dog was not killed until 31 days after feeding.

The 85 parasites obtained were no larger, nor did they have more eggs, than those from the first dog. A cat and a domesticated ferret, parasite-free by fecal examination, were also fed metacercariae from mink lung, and adult *Alaria* similar in appearance to those mentioned above were obtained in great numbers, 215 from one ferret.

Six mice which had been infected with metacercariae by being given the mesocercariae either from experimentally infected tadpoles or from naturally infected frogs were fed to another parasite-free ferret. The mesocercariae were removed from the tadpoles or frogs and put either on a small piece of moistened bread which, if the mice had been without food for a day, was readily eaten by them, or they were given to the mouse by medicine dropper. Parasites of the genus *Alaria* were obtained from the intestine of this ferret also.

The conspecificity of the adult *Alaria* from minks, weasels, ferrets, cats, and dogs was established by a careful comparative study involving the examination of a series of whole mounts of worms from each host and a comparison in tabular form of the measurements of their significant organs. Because of its size this table cannot be published. The measurements of representatives from all these hosts present a consistent and uniform series. The parasites from one mink and one weasel were dead and contracted when received, hence the average measurements for these two groups are somewhat low. All other worms were living up to the moment of fixation, and were treated as described in the section on material and methods. Throughout the series the relative measurements of the various organs and parts are in accord, so that in smaller individuals the measurements of all organs are relatively smaller. There is some indication, as shown by the series of measurements, that the trematodes from large hosts may be somewhat larger, although not significantly, than those from small hosts. The worms grown in the two ferrets are perhaps some of the most crucial of the series, as one ferret received metacercariae from mink, and the other metacercariae from mice. Comparison of measurements given for the parasites from these two hosts show remarkable similarity in size range, and when these are compared with measurements of parasites from weasel no. 1 and mink no. 1 there is also a notable conformity. The average measurements of the parasites from the ferret that had been fed mice are almost identical with those of the general total of all the worms measured, indicating that the parasites experimentally raised through two of the larval stages are typical of the species.

All whole mounts were examined for general conformity to the characters of the species in as far as they could be determined from such mounts, and no discrepancies were found, so that it may be safely concluded that worms from these diverse hosts all belong to the same species, the mink and weasel being the natural hosts. It may further be concluded that the

species is not particularly specific in its choice of hosts, for it grows equally well in such experimental hosts as cats, dogs, and ferrets, representatives of three families.

Concerning the normal transfer of the respective stages of this life cycle from one host to the next, practically nothing is known, because of the inadequate knowledge of the food habits of the series of hosts involved in this life cycle under natural conditions. The easiest step is the entrance of the cercariae into the tadpole or frog by penetration. Those frogs having the longest larval stages and those which are most aquatic should on *a priori* grounds be the ones most heavily infested, an assumption which has been found true, for adult *Rana clamitans* and *R. catesbeiana* usually had far more mesocercariae than other frogs.

The mesocercariae reach their full development in 15 to 21 days, depending to some extent on the number present. Some tadpoles died within a day after exposure to the cercariae. Similarly Blochmann (1910) and Van Haitsma (1931b) wrote of the killing of fish by penetrating cercariae. Other tadpoles lived for over a week after exposure before dying. When these were opened the whole coelom was sometimes found to be filled with mesocercariae, a condition which very likely caused the death of the host. In extreme infestations the parasites were usually somewhat smaller than from less heavily parasitized hosts, even for identical periods.

The longevity of the mesocercariae is unknown and therefore the possibility of their being carried over from one year to the next is only supposed; yet it seems entirely possible. The only frog which showed atrophic black cysts in any numbers was this one large bullfrog which was so filled with cysts that the flesh had a speckled appearance. However, the transparent viable cysts numbered many times the dead ones. In the laboratory infected tadpoles have been kept for several months, when all the larvae seemed to be alive and in good condition. Therefore, these black cysts of the bullfrog are interpreted as being old. The frog was very large and must have been several years old, but whether some of the parasites had persisted from the tadpole stage it is difficult to say.

Minks and raccoons eat frogs and tadpoles, a fact which easily explains the large numbers of metacercariae found in the lungs of some of them, but there are no similar observations to account for the metacercariae in the mice in nature. A tadpole given to a hungry laboratory mouse disappeared within an hour; apparently the mouse had eaten it. The first rats were infected when they ate flesh of a frog containing mesocercariae. Mice have been known to eat reptiles or portions of them. I have received verbal report of mice which, when placed in cages as food for rattlesnakes, were not immediately eaten by the snakes, but ate off the rattles of the snakes instead, and of mice which attacked and killed lizards in a pen. During the summer many small pools dry up leaving many tadpoles stranded thus al-

lowing mice to get at them. Cort (1918) reported the feeding of tadpoles, infected with *Agamodistomum marcianae*, to snakes and the transfer of the parasites from the tadpoles to the snakes without undergoing a perceptible change. In fact *Agamodistomum marcianae* was first described from a garter snake by La Rue (1917). No tadpoles infected with the present species of mesocercariae were fed to snakes, but snakes have been examined which had these mesocercariae in the fat bodies. It is possible that mice eat tadpoles, dead frogs and snakes and thus become infected. The shrews examined had no metacercariae in the muscles but if more were examined perhaps they might also be found to harbor the parasites, for they are more commonly found near water than mice, and are also more voracious.

That the larvae are tenacious of life and can live for some time after the death of their hosts is shown by finding the living and active meso- and metacercariae in the mink carcasses sent into the laboratory and in some cases examination must have been two, three or more days after death. The parasites were dead only in putrid or frozen carcasses. The larvae after removal from the host and without cysts remained in good condition in normal salt solution for four or five days if kept in the refrigerator.

Weasels and minks by eating infected mice receive the metacercariae which become adult *Alaria* in the intestines. There is considerable question as to the fate of the metacercariae which occur in the mink. Ameel (1931) has reported several affirmative replies to inquiries sent to trappers of mink concerning the eating of mink carcasses by dogs. These replies are of significance to the present investigation as dogs have been experimentally proved to be satisfactory hosts of this parasite. The general opinion seems to be that dogs will not eat mink; however, Ameel secured a few reports of the eating of mink carcasses by young or very hungry dogs. A cat kept in the laboratory willingly ate the whole thoracic region, including the lungs of a mink. Two other cats refused to eat any part of a mink carcass, and a ferret refused to eat a mink lung. Since all of these animals were well fed starvation was not a factor. No information is available as to whether minks are cannibalistic, either killing and eating another mink or eating the body of a dead mink. Since dogs serve as suitable hosts it is entirely probable that foxes or coyotes also serve, for both eat mice in considerable numbers.

Knowledge concerning the food relations of these various animals is not sufficient to interpret the findings connected with this life history in an entirely satisfactory manner. It is important to point out here that considerable investigation of food habits of several animals, especially small mammals, can profitably be carried on by parasitologists; for when the food interrelationships of certain animals are known life histories which have baffled investigators for years may be solved. It is wise to look beyond the obvious food relations and not to judge as to the habits of animals by the habits of pets or caged animals whose tastes are often perverted. If this

broader view be taken many life histories will be worked out despite their seeming absurdity when first proposed.

The length of life of the adult parasites is unknown. A cat remained infected two and one-half months as shown by the finding of eggs in the feces. The parasites may live much longer than this, but observation on this animal had to stop.

A brief resumé of this life history is as follows: The cercariae are produced in sporocysts in snails, *Planorbula armigera*, and emerge into the water, and within a day must penetrate into the tissue of a frog or tadpole, in which they take three weeks to become fully grown mesocercariae. When the tadpole is eaten by a mouse it is nine weeks before the metacercariae are matured in the muscles or lungs. Ten days after the infected mouse is eaten by a mink or weasel the adult parasites are sexually mature in the intestines.

MORPHOLOGY OF LIFE HISTORY STAGES

CERCARIA

Historical Review. Of the large number of described furcocercous pharyngeate cercariae the further development of only a few have been followed to the metacercarial stage. Of these only one other is known to develop into a mesocercaria in tadpoles. This is *Cercaria marcianae* (La Rue) described by Cort and Brooks (1928). All the known mesocercariae (*vide* section on mesocercariae) have four penetration glands anterior to the acetabulum. The same arrangement of penetration glands is shown by Cort and Brooks (1928) for *Cercaria marcianae*. The cercaria of *Alaria mustelae* has four penetration glands so situated as almost to surround the acetabulum (Fig. 5). Ruzskowski (1922) in his experiments with *Alaria alata* secured cercariae from snails which he had infected with miracidia. Since his figure of *Cercaria alata* lacks details no comparison can be made.

Other cercariae having four penetration glands anterior to the ventral sucker and whose later development is known are: *Cercaria A* of Szidat, *C. tarda*, and *C. douglasi* Cort. Mathias (1925) demonstrated that *C. tarda* became *Tetracotyle tarda* in snails and in the definitive host developed into *Strigea tarda* Steenstr. Szidat (1929a) considered the latter to be synonymous with *Cotylurus cornutus* Rud. Van Haitisma (1931a) proved that *C. douglasi* penetrated into snails where it became a tetracotyle which he would identify as *Tetracotyle flabelliformis* Faust (1917), and when fed to ducks matured as *Cotylurus flabelliformis*. Szidat (1924a) showed experimentally that his *Cercaria A* upon penetration into snails developed into *Tetracotyle typica* Dies. In the light of these facts it is not surprising that Cort and Brooks (1928) suggested that the adult of *C. marcianae* would very likely be closely related to the genus *Strigea* using this generic name in the broad sense employed before its revision by Szidat (1929b).

Description. The cercaria of *Alaria mustelae* came from snails, *Planorbula armigera*, collected from a pool southeast of Ann Arbor, Michigan. Infected snails were so scarce that none could be sacrificed to examine the living sporocysts. At the death of the snails examination showed that the digestive gland was filled with long, slender, unbranched sporocysts.

The cercariae emerge from the snail in such great numbers as to make the water cloudy. The cercaria comes to rest with body down and tail furcae up and well spread. As the cercaria gradually sinks downward it swims a short distance to regain the former level, swimming always with the tail foremost. When disturbed the cercaria may continue swimming for some little time. In the resting state the cercariae are evenly distributed throughout the water in the container. When old the cercariae settle to the bottom but never creep on the bottom by means of the suckers as some cercariae do.

The cercariae are very small and when only a few are present in a bottle of water they are easily overlooked.

TABLE III
MEASUREMENTS IN MILLIMETERS OF 15 CERCARIAE FIXED IN HOT 10% FORMALIN

	Body		Tail stem		Furcae	Oral sucker		Acetabulum	
	length	width	length	width	length	length	width	length	width
Average	0.101	0.039	0.165	0.031	0.146	0.025	0.021	0.016	0.016
Minimum	0.083	0.035	0.154	0.028	0.14	0.022	0.018	0.013	0.014
Maximum	0.112	0.043	0.173	0.033	0.151	0.03	0.025	0.019	0.018

The anterior part of the body is spined (Fig. 5). A narrow area directly around the mouth is without spines. This circumoral spineless area is surrounded by a number of rows of rather large retrorse spines which cover two-thirds the length of the sucker. From here posteriad are smaller spines gradually spaced farther apart until they disappear at a level slightly anterior to the ventral sucker. The opening of the acetabulum, when expanded is surrounded by a single row of spines and when contracted by two irregular rows. The tail stem is spineless but the furcae are irregularly spinose. The tail stem has the usual long hair-like structures, common to strigeid cercariae, projecting from it. There are no caudal bodies. Midway between the oral and ventral suckers in the lateral fields are the inconspicuous, unpigmented eye spots (Fig. 5).

The mouth is slightly subterminal in the oral sucker, prepharynx short, pharynx small and muscular, esophagus rather long, the intestinal ceca extending a short distance posterior to the acetabulum. The oral sucker is slightly larger than the ventral, a character which holds for all the stages of development. On either side of the ventral sucker there are two unicellular penetration glands (Fig. 5) filled with a finely granular substance. The

long ducts of these glands follow a sinuous course anteriorad, penetrate the oral sucker and open on either side of the mouth in the circumoral spineless area. The primordium of the reproductive system is present as a small mass of cells anterior to the excretory bladder (Fig. 5).

The excretory system of *Cercaria mustelae* (Fig. 12) follows the plan usual for strigeids. The limbs of the excretory bladder extend anteriorad to the posterior margin of the ventral sucker where they form two or three irregular convolutions before bifurcation as the anterior and posterior collecting ducts (Fig. 12). On each side the anterior collecting duct has three flame cells attached and the posterior two in the body and two in the anterior part of the tail stem. The bladder divides at the bifurcation of the tail and extends almost to the tips of the furcae before opening to the exterior through small pores (Fig. 5).

Discussion. *Cercaria marcianae* is the only other cercaria known which upon penetration into a tadpole is known to become a mesocercaria. Several characters clearly distinguish it, as described by Cort and Brooks (1928), from *C. mustelae*. The flame cell patterns of the two forms are fundamentally alike but in *C. marcianae* each original flame cell has already divided to form two so that there are three sets of two each connected to the anterior collecting duct and the posterior tubule has two sets of two flame cells in the body and one in the tail attached to it, while in *C. mustelae* the flame cells have remained undivided except for those in the tail stem. The flame cell formulae are: for *C. marcianae* $2[(2+2+2)+(2+2+(2))]$, for *C. mustelae* $2[(1+1+1)+(1+1+(2))]$.

The position of the penetration glands is another difference between these cercariae. In *C. marcianae* these glands are anterior to the acetabulum while in *C. mustelae* the glands are arranged more laterally to the ventral sucker (Fig. 5). *C. marcianae* has a number of rows of spines surrounding the opening of the ventral sucker as compared to the irregular double row found on the contracted sucker of *C. mustelae*. The body is only partially spined in *C. mustelae* in contrast to the spination of the entire body in *C. marcianae*. Some of the characters common to the two forms are: small size, four penetration glands, unpigmented eye spots, two pairs of flame cells in the proximal portion of the tail stem, no caudal bodies in the tail stem, no forward pointing spines in the circumoral spineless area, no commissure connecting the two sides of the excretory system. The above comparison plainly shows that these two species are distinct but nevertheless closely related.

Other fork-tailed pharyngeate cercariae having four penetration glands but differing in certain important respects from *C. mustelae* are *C. furcicauda* Faust (1919), *C. Indicae* I. Sewell (1922), *Cercaria A* Szidat, Dubois (1928), *C. sanjuanensis* Miller (1927), *C. helvetica* XIV and *C. helvetica* XXIX Dubois (1928), *C. letifera* Fuhrm. Dubois 1928, and *C. tenuis* Miller (1926).

THE MESOCERCARIA

Historical. The term *Agamodistomum* as applied to strigeids was interpreted by Hughes (1928b) to include those larval strigeids which have in common a similar body form and retain certain of the cercarial structures, such as the penetration glands, after their entrance into the secondary host. As mentioned in the introduction, a prolonged agamodistome stage is essential to the life cycle of *Alaria mustelae* and therefore for convenience a new name is desirable to distinguish it. The term agamodistomum simply means larval distome and is applicable to any immature distome without implication as to its possible systematic position. The name mesocercaria is an appropriate designation for this definite prolonged intermediate stage which occurs in the secondary host between the cercarial and the definitive metacercarial phases in the life cycle of those trematodes having four hosts.

Gastaldi (1854) described the first mesocercaria from *Rana esculenta* under the name *Distoma tetracystis*. A similar form, *Distomum putorii*, from cysts in the throat muscles and esophagus of *Foetorius putorius* was briefly described by Molin (1858). Von Linstow (1875) redescribed it more fully and called attention to its similarity with *Distoma tetracystis* from the frog. In fact he said, "Ich habe keine Unterscheide finden können." It is of interest to point out here again that the only known *Diplostomulum* from mammals also comes from *F. putorius*, a fact which might be interpreted to mean that one is the earlier stage of the other. La Rue (1917) described *Agamodistomum marcianae* from *Thamnophis marciana* (Baird and Girard) and *T. eques* (Reuss), taken in Texas. The next year Cort (1918) reported a similar form from tadpoles at Douglas Lake, Michigan, which he identified as *marcianae*. Cort demonstrated by feeding infected tadpoles to snakes that these flukes passed through the intestinal wall of the snake and into the tissues without undergoing any perceptible morphological change. The proof of the relationship of these larval trematodes to the Strigeids was established by Cort and Brooks (1928). Cort (1918) had already showed that certain fork-tailed, pharyngeate cercariae upon penetration into tadpoles become mesocercariae which he identified as *Agamodistomum marcianae*. *Agamodistomum ordinata* (Nicoll, 1912) Hughes (1928b) from North American striped snakes closely resembles *A. marcianae*. In the inspection of meats for *Trichinella* another mesocercaria was discovered in the muscles of pigs in Saxony. This form, *Agamodistomum suis* Stiles, was first described by Duncker (1881). Stiles (1900) reported larval trematodes in the muscles of swine in this country but did not definitely identify them with *A. suis*.

Description of Mesocercaria. The mesocercaria of *Alaria mustelae* has the appearance of an enlarged cercaria body without a tail. It is elongated, oval, flattened dorso-ventrally, 0.24–0.34 mm. long, and 0.143–0.205 mm. wide. In some fixed specimens there is a slight indication of a hindbody. The cuticula anteriorly is covered with fine spines, more densely distributed

and larger over the anterior two-thirds of the oral sucker, and diminishing in size and number per unit area from here to the level of the anterior margin of the first pair of penetration glands (Fig. 4). This distribution resembles that found in the cercaria. The oral sucker, 0.052–0.08 mm. long and 0.042–0.07 mm. wide, is slightly larger than the centrally located acetabulum which is 0.04–0.052 mm. long and 0.048–0.066 mm. wide. Its opening is armed with spines, which appear as two irregular rows on the contracted sucker, and as a single row on the expanded sucker.

The mouth is sub-terminal, the prepharynx short, pharynx small, esophagus short, and the intestinal ceca extend posteriad a short distance beyond the penetration glands (Fig. 4). The two pairs of voluminous, unicellular penetration glands with finely granular contents almost completely surround the ventral sucker (Fig. 4). The ducts of both pairs of glands are of about equal length, but the anterior ducts only are coiled. The adjacent ducts run along side by side, penetrate the oral sucker and open beside the mouth.

A small mass of cells, the fundament of the reproductive system, lies between the two limbs of the excretory bladder. The excretory system will be considered in a later section.

Discussion. Mesocercariae have so few characters useful in specific diagnosis, especially in fixed specimens, that it is difficult to determine to which species they belong. I have found that the penetration glands persist for some time after entrance into the third host even after growth of the larva. Therefore, specimens taken from the same mammal may vary considerably in size depending upon the length of sojourn in the host and may be much larger than those taken from the second intermediate host. This fact makes measurements of comparatively little value in deciding conspecificity of mesocercaria taken from second and third intermediate hosts.

Agamodistomum la-ruei Hughes (1928b) from the lungs of a raccoon from Douglas Lake, Michigan, is of the same size as the mesocercaria of the present species. However, there are several reasons for considering them to be not the same. The measurements given of *A. la-ruei* show an acetabulum that is larger than the oral sucker while this organ is smaller than the oral sucker in the mesocercaria of *A. mustelae*. In fixed and mounted, as well as living, mesocercaria of *A. mustelae*, the penetration glands are very large and surround the ventral sucker in a characteristic manner. The ventral sucker is usually in the middle of the body. In the type material of *A. la-ruei* the glands are smaller, situated anterior to the middle of the sucker, which is anterior to the middle of the body. The germ cells of *A. la-ruei* appear to be a strand of loosely arranged cells while in *mustelae* the germ cells form a solid mass.

The other American species are an agamodistome from swine and *Agamodistomum marcianae* (La Rue). The American mesocercaria from

swine has not been definitely assigned to *Agamodistomum suis* of Europe. According to Leuckart (1889), *A. suis* is 0.5 mm. long, which is considerably longer than the mesocercaria of *Alaria mustelae*, and his figure shows three masses of cells in the posterior part of the body which are interpreted to be distinct reproductive fundaments. Although the development of the reproductive system is much advanced in the metacercaria of *A. mustelae* no indication of development of this system was seen in the mesocercaria. During metamorphosis one of the first structures to appear is the holdfast organ, while the penetration glands or their remnants persist for some time after its formation. Thus *A. suis*, which is already in the mammalian host, may have been observed during its transformation, and the center and most anterior mass of cells might be interpreted to be the beginning of the holdfast organ.

The mesocercaria of *A. marcianae* (La Rue) has been the most fully described of all the known species and the only one whose excretory system has been studied. This was done by Cort (1918), and later Cort and Brooks (1928) showed the derivation of the flame cell pattern of the mesocercaria from that of the cercaria. The excretory pattern of *marcianae* is much more advanced than that found in the present species. Another difference distinguishing these two species is size, *marcianae* measuring 0.354–0.459 mm. by 0.184–0.286 mm., while the maximum length of the mesocercaria of *Alaria mustelae* is 0.34 mm., about equal to the minimum for *marcianae*. What has been said of the position of the penetration glands in *la-ruei* also applies here as *marcianae* has four glands anterior to the ventral sucker. Cort (1918) showed *marcianae* to have several rows of spines around the ventral sucker while *mustelae* has but a single row. La Rue (unpublished research) has not been able to get *marcianae* to develop beyond the mesocercaria stage in mice.

Mesocercaria *putorii* (Molin) Stossich as described by von Linstow (1875) is a very large form 0.9 mm. long by 0.7 mm. wide with oral and ventral suckers of equal diameter. All these mesocercariae are larger than that of *A. mustelae*, and this is of interest for the adult of this species is smaller than any other known species of *Alaria*.

THE METACERCARIA

Historical. Hughes (1929a) reviewed the known species of diplostomula making repetition here unnecessary. The only species recorded from a mammal is *Diplostomulum putorii* (von Linstow) Hughes which von Linstow found encysted in the wall of the esophagus and intestine of *Foetorius putorius*. Other warm blooded hosts reported for these larvae have been birds.

Tetracotyles have been reported from mammalian hosts by von Linstow, namely: *Tetracotyle soricis* von Linstow (1877: 191) from *Sorex vulgaris*, and *T. foetorii* von Linstow (1876: 1) from *Foetorius putorius*. The

available knowledge is very limited concerning these few larvae from warm blooded hosts. Some of them may be stages of *Alaria*.

Description. The metacercariae of *Alaria mustelae* have been found in muscles and lungs of mice, *Peromyscus leucopus noveboracensis*, mink, *Mustela vison*, and raccoon, *Procyon lotor*, as natural infections and have been grown experimentally in laboratory-raised rats and mice.

The diplostomulum type of metacercaria of *A. mustelae* consists of a thin, leaf-like, elongated, ventrally concave forebody, and a small cylindrical but well defined hindbody (Fig. 2). The forebody possesses all the characters of the adult, but is smaller.

TABLE IV
MEASUREMENTS IN MILLIMETERS OF TEN PRESERVED METACERCARIAE MOUNTED *IN TOTO*

	Minimum	Maximum	Average
Total length	0.492	0.716	0.613
Hindbody, length	0.116	0.17	0.15
Hindbody, width	0.134	0.196	0.168
Oral sucker, length	0.062	0.089	0.075
Oral sucker, width	0.062	0.071	0.068
Pharynx, length	0.062	0.089	0.07
Pharynx, width	0.044	0.62	0.053
Acetabulum, length	0.053	0.062	0.06
Acetabulum, width	0.062	0.071	0.065
Holdfast, length	0.134	0.196	0.163
Holdfast, width	0.08	0.116	0.102

The forebody may retain its concavity, the foliaceous margins their ventral infolding even in preserved and mounted specimens. The oral sucker is usually prominent with the mouth opening slightly subterminal. In the oral sucker of even the most mature metacercariae some remnants of the ducts of the penetration glands can still be seen, both in living and mounted specimens. The sucking cups, lateral to the oral sucker, are usually drawn in. The acetabulum, situated well anterior on the ventral surface of the forebody, is smaller than the oral sucker (Fig. 2).

The oval holdfast organ is capable of a variation in expansion almost as great as in the adult. However, in no specimen was it so extensive as to cover the acetabulum and pharynx. The cuticula of the forebody is covered with many closely set, retrorse spines, largest and most densely arranged in the anterior portion of the body. The hindbody is relatively small, cylindrical, and attached to the postero-dorsal surface of the forebody. Oral sucker, a large muscular pharynx, very short prepharynx and esophagus, and intestinal ceca which extend almost to the posterior end of the hindbody constitute the digestive system. The excretory system will be considered in detail in the section dealing with development. The more mature metacercariae present a precocious development of the reproductive system. In the previously described forms of diplostomula the reproductive system

is represented by a single fundament, or at most a lobed primordium suggesting the formation of the later organs. Hughes (1929a) reported the presence of a bursa copulatrix in *Diplostomulum corti*.

The metacercaria has all the parts of the sexual apparatus of the hermaphroditic adult in correct form and arrangement (Fig. 3). However, the two testes are relatively much smaller than in the adult. The vasa efferentia could not be distinguished and only a portion of the narrow vas deferens was seen. A tubule anterior to T₁ was interpreted to be the beginning of the seminal reservoir. The empty seminal vesicle lacked the voluminous size usually seen in the adult, and appeared as a more or less straight tube extending in an antero-posterior direction.

The parts of the female genital system are all present and occupy the same relative positions as in the adult, but they are obviously immature. The ovary is relatively small and the oviduct very wide at its origin. The vitelline reservoir is represented by a narrow tube, and a small mass of cells grouped about the distal portion of the oviduct marks the location of the future Mehlis' gland and oötype. The uterus is narrow and straight, and its anterior portion just comes into contact with the holdfast organ without entering it. The vitellaria are represented by groups of large deeply staining cells, in the parenchyma of the lateral fields of the forebody. These cells have large nuclei and prominent nucleoli. There is no indication of the brownish pigmentation of the vitelline cells of the adult, hence these cells are invisible in living worms and whole mounts.

Discussion. Several metacercariae are known which show a considerable differentiation of the reproductive organs. Some species are so precocious as to be paedogenetic or, according to Giard (1887), progenetic. According to Dollfus (1929) the height of progenetic development is reached in such a form as *Ratzia parva* (Stossich) in which eggs containing miracidia are produced by the encysted metacercaria. He suggested that this may be a step toward the elimination of the final host. Development in the metacercaria of *A. mustelae* is not so advanced, although it is greater than for any other known metacercaria belonging to the Strigeoidea.

Within the muscle the metacercariae of *A. mustelae* are encased in thin-walled cysts between the muscle fibers. A series of seven cysts taken from an experimentally infected rat, without cover glass pressure, had an average length of 0.852 mm. and width of 0.526 mm. The shortest cyst was 0.75 mm. long and the longest 1.005 mm., the narrowest 0.48 mm. and the widest 0.6 mm. As the cyst is considerably larger than the metacercaria there is sufficient room for this active larva with its rhythmic, wave-like motion to move about. The cyst is flexible and its shape responds to the movement of the metacercaria within. Changes in shape occur more readily perhaps when the cyst is free than when embedded between the muscle fibers of the living host.

In the lungs there is practically no encapsulation around the metacercaria. The presence of the organisms in the lung produces a marked atrophy of the alveoli between the metacercaria and the pleura. This is perhaps caused by pressure, brought about by the growth of the parasite, which is considerable from the time of its entrance as a mesocercaria until the full maturity of the metacercaria, by movement of the worm within its cavity, and the subsequent disturbance of blood circulation.

The cyst found around the metacercaria in the muscle seems to be entirely of host origin, for it has the fibrillar appearance of connective tissue. Other facts lending support to this view are: the lack of a true cyst of parasite origin, a characteristic of diplostomula, the absence of cystogenous glands in mesocercaria and developing metacercaria and the lack of cyst formation in the lung.

As mentioned above the only other metacercaria found in a mammal is *Diplostomulum putorii* (von Linstow) Hughes (1929a) from the wall of the esophagus and intestine of *Foetorius putorius*. The figure and description of this metacercaria as given by von Linstow shows a form sharply pointed anteriorly, without lateral sucking cups or a hindbody, and a small globular holdfast organ. Many spherical calcareous concretions are thickly distributed in the parenchyma. These characters do not fit the present species which has a broad anterior end, lateral sucking cups, a hindbody, an elongated holdfast organ, and no very great number of calcareous concretions. The description of *D. putorii* more closely resembles another undescribed metacercaria which was occasionally found in the muscle and lungs of mink when examination was being made for *D. mustelae*. It is without lateral sucking cups or appendages, has a small holdfast organ and very many calcareous concretions. These metacercariae were never found in sufficient numbers to be of use in feeding experiments. The smallest ones found give no indication of the cercarial characters. Nothing being known of its early or later development a determination of its relationships must await a future investigation. A clue is furnished by the discovery in the intestine of mink of a very few adult worms without lateral sucking cups and a less elongated holdfast than that of *A. mustelae*. Hall and Wigdor (1918) described *Alaria michiganensis* from the intestine of dogs as pointed anteriorly and without lateral appendages. Whether there is any relationship between these metacercariae and either of these adults without lateral projections can be at present only a matter of conjecture.

ADULTS

Historical. Goeze (1782) found the first trematodes of the genus *Alaria* in the intestine of a fox and named it *Planaria alata*. The generic name *Alaria* was given to this species by Schrank (1788). Since that time many investigators have considered this species from both morphological and

taxonomic standpoints. Krause (1914) published the last and most complete work on *Alaria alata*, with a resumé of the literature and an adequate description of its structure. He recognized the priority of the name *Alaria* Schrank, which almost all previous investigators had failed to do.

The first North American members of the genus *Alaria* were found in the intestines of dogs by Hall and Wigdor (1918) at Detroit, Michigan, while experimenting with anthelmintics. They distinguished two species, *Hemistomum americana* and *H. michiganensis*. These were placed in the genus *Alaria* by Railliet (1919). *A. arisaemoides*, from *Vulpes fulva*, was described by Augustine and Uribe (1927). La Rue and Townsend (1932) described *A. nasuae* from *Nasua nasica*, and La Rue and Barone (1932) described *A. oregonensis* from the intestine of a coyote.

All the seven species of *Alaria* so far described, except *Alaria nasuae*, were derived from the carnivore family Canidae including wolves, foxes, domestic dogs, and related species. *A. nasuae* occurred in *Nasua nasica*, family Procyonidae. The present species has its type host in the genus *Mustela*, family Mustelidae.

Specific Diagnosis. *Alaria mustelae*—*Alaria*; adults small 0.82–1.74 mm., average 1.25 mm.; forebody foliaceous and scoopshaped, 0.94–1.1 mm., average 0.78 mm. long, not separated from hindbody by a deep groove; hindbody cylindrical, $0.241\text{--}0.716 \times 0.259\text{--}0.483$ mm., average 0.483×0.34 mm.; oral sucker $0.062\text{--}0.116 \times 0.062\text{--}0.107$ mm., average 0.082×0.082 mm., larger than acetabulum, latter with diameter of 0.053–0.089 mm., average 0.07×0.072 mm.; pharynx large, $0.071\text{--}0.125 \times 0.062\text{--}0.116$ mm., average 0.106×0.078 mm.; holdfast organ $0.25\text{--}0.58 \times 0.116\text{--}0.34$ mm., average 0.379×0.211 mm., very contractile; lateral sucking cups present; entire forebody spined; ovary 0.107–0.214 mm. wide, average 0.152 mm., anterior to testes; Mehlis' gland lateral to anterior testis; posterior testis 0.187–0.36 mm. wide, average 0.258 mm., larger than anterior testis; anterior testis 0.152–0.32 mm. wide, average 0.189 mm., sometimes appearing to be in forebody; anterior loop of uterus enters holdfast organ; eggs in uterus few, $0.089\text{--}0.134 \times 0.062\text{--}0.089$ mm. Habitat, intestine of mink and weasel. Metacercaria in muscles and lungs of mice, mink and raccoon. Mesocercaria in frogs and tadpoles. Cercaria from sporocysts in the snail, *Planorbula armigera*.

Description. *Alaria mustelae* is a small strigeid trematode with the characters of the genus. It is found in nature in the intestines of the mink, *Mustela vison mink*, and weasel, *Mustela noveboracensis noveboracensis*, and was grown experimentally in a cat, dogs and ferrets. In all the hosts the trematodes were found in the anterior third of the small intestine closely adhering to the mucosa. They were freed from the mucosa only after hard shaking of the intestine in salt solution, or by scraping the inner intestinal surface with a blunt instrument.

External appearance. The external features of *Alaria mustelae* are much like those of the other species of the genus (Fig. 1). In life it is remarkably transparent and mobile, changing its shape and length with the greatest ease by muscular contraction and relaxation of fore- and hind-bodies (Fig. 1). The two regions of the body are not sharply marked off by a groove, nor does the forebody have a prominent ventral overhanging lip. The forebody is concave ventrally, and usually longer and much broader than the hindbody. The so-called lateral sucking cups are rarely everted as processes. The tissue lining the lateral cup is glandular. Its structure has been discussed in detail by Krause, 1914: 106-111, La Rue, 1927: 28, and both its structure and its function by Van Haitsma (1931b) in his work on *D. flexicaudum*.

The holdfast organ is conspicuous and varies greatly in form. It may be completely drawn in, showing only as a longitudinal slit posterior to the acetabulum, but in stained specimens the tissue of the holdfast organ stains more deeply and its extent is therefore observable. When everted the size of the holdfast is not constant, it may be no longer than the original longitudinal groove or it may be so expanded as to cover the acetabulum and pharynx and fill the whole concavity of the forebody. The protruded holdfast organ usually shows a median longitudinal depression (Fig. 1).

The acetabulum is almost circular, a little wider than long, and slightly smaller than the oral sucker. In no specimen have I seen the acetabulum protruded, nor in living worms any activity of this organ which might indicate that it serves as an important organ of attachment in the adult. The entire forebody is densely covered with fine retrorse spines, those of the anterior portion being slightly larger and set more closely together than the posterior.

The cylindrical hindbody may lie in a straight line with the forebody or it may appear to be attached somewhat dorsally to the forebody. This appearance may be due to a dorsal flexure of either the hind- or fore-bodies. The excretory pore is located at the posterior tip of the hindbody and dorsal to it is a depression that marks the genital atrium and bursa copulatrix.

The digestive system consists of the usual parts, the only peculiarity being the large size of the pharynx and the shortness of the esophagus (Fig. 1). The excretory system will be described in connection with the discussion of the development of this system.

The male reproductive system (Fig. 10) is arranged much like that of *Alaria alata* as shown by Krause (1914). The anterior testis lies well forward of the middle of the hindbody. It does not have the characteristic horseshoe shape but is somewhat lobed, the left posterior portion being sharply cut off, making room for the female ducts, Mehlis' gland and vitelline reservoir. The vas efferens arises a little to the right of the center on the ventral surface of the testis, ascends and bends to the right around the

testis to enter the seminal reservoir which is anterior to the testis (Fig. 6).

The posterior testis is larger than the anterior and is located directly posterior to it. There is a deep groove down the ventral longitudinal surface which increases in dimensions posteriorly and dorsally so that in cross section it appears here as two separate structures. The vas efferens of this testis arises on its mid-ventral face, ascends dorsal to the vas deferens to the anterior level of the testis, then after bending first to the left, then dorsad, ascends again around and over the anterior testis where it enters the seminal reservoir (Fig. 6). The seminal reservoir lies over the anterior testis ventral to the ovary (Fig. 6). The vas deferens originates from the center of the reservoir, descends, curving around the left ventral surface of the anterior testis, before it bends to the right and comes to be located directly dorsal to the descending uterus (Fig. 9). Posteriorly the vas deferens bends dorsally becoming the seminal vesicle where it swells out to fill the space between the lobes of the posterior testis. The seminal vesicle discharges through a rather long, somewhat muscular ductus ejaculatorius into the genital atrium (Fig. 10).

The female reproductive system has the usual parts. The ovary, oval from the anterior aspect, lies above the anterior testis toward the right side of the body (Fig. 11). It has never been noted on the left side. When the worm is contracted the ovary and the anterior testis sometimes appear to be drawn up into the forebody, and the ovary may seem to lie in the posterior portion of the holdfast organ if the hindbody is especially contracted. The oviduct arises from the middle of the posterior surface of the ovary (Fig. 7). Laurer's canal originates from a slightly swollen portion of the oviduct which may perhaps serve as a seminal receptacle (Fig. 11). After following a somewhat sinuous course Laurer's canal opens to the outside on the dorsal surface (Fig. 11). The oviduct extends to the left in a dorsal position, and receives the vitelline duct upon its entrance into the Mehlis' gland (Fig. 7). The oötype passes through the Mehlis' gland posteriad and dextrad, emerging as the uterus which continues to the right a short distance, then bends back upon itself, making a short transverse loop (Fig. 8) then ascends, remaining close to the left margin of the anterior testis (Fig. 7) and continues anteriad into the holdfast organ (Fig. 11). The extent of the penetration of the uterus into the holdfast organ seems to depend on the number of eggs in the uterus, for it was noticed that the larger the number of eggs the greater the number of uterine coils in the holdfast organ. The descending limb of the uterus passes posteriad in the mid-ventral plane to empty into the genital atrium just ventral to the ejaculatory duct (Fig. 11).

The vitelline follicles are distributed in the forebody from the acetabulum posteriad, with many in the holdfast organ. They extend in the hindbody to the posterior margin of the ovary. The vitelline ducts run mesad to the intestinal ceca to a position between the two testes where they join the

vitelline reservoir which extends ventro-dorsad between the two testes, displaced slightly to the left of the midline (Fig. 8). The common vitelline duct originates from the dorsal end of the reservoir, extends antieriad along the margin of the Mehlis' gland until it joins the oviduct (Fig. 7).

Eggs are few, fifteen being the greatest number in one individual among all the seventy specimens examined. Many worms of equal size had no eggs in the uterus, while the commonest number was two or three. Eggs are of the usual strigeid type, elliptical in shape, operculate, and yellowish brown in color. The uterine eggs are 0.089–0.13 mm. long and 0.062–0.089 mm. wide while eggs from the feces are 0.107–0.125 mm. long and 0.071–0.08 mm. wide. The average size of the 60 uterine eggs, 0.11×0.065 mm., is slightly smaller than the average of the 15 eggs from feces, 0.119×0.073 mm., the latter having a less varied size range.

Alaria mustelae has been carefully compared with previously described species of the genus. All, except *A. americana* Hall and Wigdor and *A. michiganensis* Hall and Wigdor, are markedly larger and thus can readily be distinguished. Although the measurement ranges of *A. americana* and *A. mustelae* overlap slightly, the former is on the whole larger, for the average measurements of several structures of *A. mustelae* are about equal to the minimum of *A. americana*. The greatest differences lie in the measurements of the pharynx and the hindbody.

The drawings of *A. americana* are not sufficiently detailed to warrant a comparison of structures. According to Hall and Wigdor the two testes are located in the lateral fields of the hindbody, apparently a misinterpretation.

Alaria michiganensis described at the same time as *A. americana* by the same authors and from the same host is distinguished by the absence of lateral processes. Almost every measurement of *A. michiganensis* is within the range given for *A. americana*. From the wild mink an occasional adult trematode has been taken which lacked sucking cups and appendages. Also in the muscle and lungs of mink a few metacercariae were found without lateral sucking cups. These last were never found in sufficiently large numbers to make feeding experiments possible. Whether one is the larval stage of the other is, therefore, unknown. Until their material or similar material can be restudied *A. michiganensis* must be retained as valid.

DEVELOPMENT OF ORGANS THROUGH LIFE HISTORY STAGES

As mentioned in the section on "Material and Methods," a series of developmental larval stages, from the mesocercaria to the mature metacercaria, found in the lungs of the mink made possible a detailed study of the formation and the later advances in growth of several of the organs. This material has been particularly favorable for such study since the mature metacercaria has all the organs of the adult but in a less mature form.

Thus they are simpler and more easily seen, and the process of development is so slow that with a few minks containing large numbers of worms it is usually possible to have the whole developmental series represented in closely graduated steps. Certain organs such as the oral sucker have not been dealt with since they undergo little change, or they are considered in connection with other organs.

Pharynx. The fully developed pharynx of *Cercaria mustelae* is small as compared with the oral sucker. In the mesocercaria these two organs grow but little, however, the pharynx has grown somewhat more rapidly than the other. During the transformation of the mesocercaria into the metacercaria the pharynx undergoes rapid increase and becomes 0.07 mm. long, about equal to the 0.075 mm. length of the oral sucker, but it is not as wide as the latter. The average size of the pharynx in the adult is 0.106×0.078 mm. as compared with 0.082×0.082 mm. for the oral sucker, which shows another marked increase in the size of the pharynx, so that in the adult it is longer, but narrower than the oral sucker and is one of the distinguishing characters of the adult.

Anterior Body Region and Esophagus. The esophagus in the cercaria is rather long, while in the mesocercaria it is relatively much shorter, and remains so through the succeeding stages. A like relation is observed in the figures of *Cercaria marcianae* by Cort and Brooks (1928) and of *Agamodistomum marcianae* by Cort (1918). This lack of growth of the anterior body region is noticeable in all the stages following the mesocercaria (Figs. 14-18).

Penetration Glands. Usually the contents of the penetration glands in cercariae are used up in the penetration of the cercarial body into the intermediate host. The presence of penetration glands in certain larval distomes in their second intermediate hosts has long puzzled students of trematodes. The reason for the existence of the penetration glands in the mesocercaria is now revealed. The cercaria of *A. mustelae* probably uses most of the secretion of the glands in entering the tadpole, but the glands, instead of atrophying as happens in species having three hosts, are regenerated, become much larger than before, thus making it possible for the parasite to penetrate the tissues of the next host. Not all the contents of the glands are used in boring through the intestinal wall and other organs of the mammalian host for the mesocercariae found in the lungs show these glands, and succeeding stages show them in successive degrees of atrophy until in the metacercaria only remnants of the ducts remain in the oral sucker.

Holdfast Organ. This is one of the first organs formed after the entrance of the mesocercaria into the mammalian host. It begins as a concentration of cells posterior to the acetabulum. Before the atrophy of the bodies of the penetration glands is complete it is a well-formed organ, at first but little

longer than wide but increasing in length and width with the growth of the metacercaria and later of the adult (Figs. 14–18).

Lateral sucking cups. A character peculiar to the metacercaria and adult is the lateral sucking cups. These do not appear in the metacercaria until after the penetration glands begin to shrink, and the holdfast organ has been formed. They begin as shallow depressions on either side of the oral sucker, after which they gradually increase in depth and develop the characteristic glandular tissue.

Spination. Another feature of the metamorphosis of the larva from the meso- to the meta-cercarial stage is the change in spination. In the cercaria and mesocercaria only the anterior portion of the body is spined, the anterior spines being larger and more closely set and distinctly separated from the others (Fig. 4). The whole forebody is spined in the mature metacercaria, the spines being more numerous and there being no line of demarcation between the large and small spines as in the mesocercaria. The explanation of these phenomena was only conjectural until it was discovered that the old spines were being shed and new ones were taking their places. The shedding of the spines is co-eval with the formation of the lateral sucking cups. With the loss of the old spines the new ones appeared in regular order from anterior to posterior. The anterior spines are largest in the metacercaria also, but there is a gradual transition in size and not an abrupt change from large to small spines as in the mesocercaria. The spines of the metacercaria are more closely arranged than in the mesocercaria, and the cuticula of the entire forebody is covered including the ventrally concave surface and the holdfast organ.

Reproductive System. Hughes (1929a) in describing *Diplostomulum corti* mentioned the presence of a bursa copulatrix and considerable differentiation of the reproductive fundament, but did not refer to either in his descriptions of the other diplostomula thus indicating that they are not of common occurrence. He did not indicate the degree of differentiation of the reproductive fundament so that no comparison can be made between *D. corti* and the metacercaria of *A. mustelae* in this respect. In all the larvae of the neascus type described by Hughes (1927, 1928a, b, and c) the reproductive system has become differentiated, so that two, three or more fundaments could be seen which, however, were not sufficiently advanced in development to allow for interpretation.

The reproductive system as found in the metacercaria of *A. mustelae* has been described above and therefore will not be repeated here, but the sequence of events in the development of the system as far as observed will be given. The first indication of the development of the genital system is the presence of the bursa copulatrix which is seen as a depression on the posterior dorsal surface. Then that portion of the reproductive primordium which is to be the ovary is separated off early. It has a somewhat trilobate

form, two lobes forming a kidney-shaped structure and the third, located in the position of the hilum, is globular. From this latter structure the oviduct later develops. The oviduct in the fully matured metacercaria is broad (Fig. 3) and in a few specimens in the anterior end of the duct two or three large cells having large nuclei and prominent nucleoli were seen. These cells were interpreted to be ova ready for passage down the oviduct. The main part of the ovary had no large cells.

The fundament from which the testes arise is at first a single broad band lying across the hindbody, from whose right anterior surface a mushroom-like structure is budded off. This is the beginning of the anterior testis. The stalk connecting the two testes becomes gradually more slender and finally apparently breaks separating the two testes. It is possible that a slender thread of the stalk remains and becomes the vasa efferentia, although this was never actually seen. The anterior testis is at first small and bean-shaped, later growing and assuming the contours found in the mature worm.

The origin of the various vessels such as the oviduct, uterus, and vas deferens was not determined. At the stage when the two testes were still connected the presence of the uterus was observed as an annulated tube, extending ventral to the other organs from the bursa to anterior of the ovary, where it loops and then descends again, but its connections could not be determined. The annulations of the uterus are caused by the prominent muscles. At this same time the ovary was apparently without an oviduct. In all specimens, but the very youngest, in which a bursa copulatrix occurs, careful observation reveals a tube extending antieriad from it which can be nothing else than the developing uterus.

Excretory System. The pattern of the excretory system of the cercaria (Fig. 12) is simple, consisting of an anterior extension of the bladder on each side which, after coiling somewhat, bifurcates into anterior and posterior collecting tubules, the anterior of which has the capillaries of three flame cells attached to it and the posterior four flame cell capillaries, two in the body and two in the tail stem. The flame cells of the tail are, of course, lost with the shedding of the tail during penetration into the secondary host. It should be noted that in *Cercaria marcianae* all the flame cells are arranged in groups of twos, but as pointed out by Cort and Brooks (1928) the fundamental pattern consists of two flame cells anterior and two posterior just as it is in *C. Indicae* I Sewell, *C. letifera* Fuhrm. and also in *C. mustelae*.

During the development of the mesocercaria there is a general lengthening and growth of the excretory system and a multiplication of flame cells, so that where one was found in the cercaria there are three in the mesocercaria, but there is no change in the basal pattern (Fig. 13). Thus the five flame cells per side in the cercaria have increased to fifteen in the mesocercaria. In comparison the multiplication of flame cells from the ten per side

in *Cercaria marcianae* to the sixty in the mesocercaria has resulted in a much more complex and advanced system.

No advance in the development of the excretory system beyond that mentioned was observed in the mesocercaria from frogs and tadpoles, but shortly after entrance into the first mammalian host, growth in this system begins by a multiplication of flame cells. Although the capillaries of numerous flame cells were seen attached to each accessory collecting duct in many specimens, no attempt was made to enumerate them since the formation of the secondary or reserve bladder system was of greater interest.

One of the most interesting features of this study was the following of the development of the anterior parts of the excretory bladder. At first the anterior coiled end of the bladder shortens, and the middle of the bladder bends mesiad (Fig. 14). The anterior part of the bent portion continues to grow ventrad and mesiad (Fig. 15). In the meantime the other organs of the metacercaria have begun their growth and there is a well differentiated holdfast organ. The above mentioned new branches of the bladder on each side of the body approach each other just posterior to the holdfast organ. Wherever they meet they fuse. As seen in some specimens fusion was at first a uniting of the anterior and posterior walls leaving an island or space between them which later closes making the two limbs continuous, and allowing a free flow of the contents from one side to the other (Fig. 17).

Shortly after this bladder has begun to form the other portions of the reserve bladder system also begin their development. The anterior end of the original primary bladder grows forward beyond the bifurcation into the collecting tubes (Fig. 15). At a point opposite the middle of the holdfast organ this anterior branch divides, giving rise to a main lateral tube which in turn divides to form the antero- and postero-lateral branches of the reserve system, and a branch which continues forward (Fig. 16). This latter gives rise to a mesiad-curving branch in the region of the acetabulum to meet a like branch from the opposite side and thus to form a median transverse commissure (Fig. 17), and a still more anterior branch, one of the secondary branches of which joins with that of the opposite side to form the anterior transverse commissure in the region of the pharynx (Fig. 17). Later secondary and tertiary divisions of all the tubules occur and the terminal tubules with their calcareous corpuscles are formed. The origin of the calcareous corpuscles was not determined. The final pattern of the excretory system (Fig. 17) with the exception of the peculiar main bladder is much like that figured for *Diplostomulum volvens* by Fraipont (1880) and for *D. browni* by Hughes (1929a).

Cross sections of young metacercaria show that the two tubular portions of the bladder extend anterior to the bifurcation. These were also seen in living whole mounts of comparable size. In cross sections of the full grown metacercaria two more longitudinal vessels may be seen than are visible in

living specimens. Occasionally when studying the living metacercariae indications of vessels running parallel to the main trunks of the bladder were noticed, but their connections could not be ascertained. The sections show four longitudinal vessels in the hindbody, two ventral and two dorsal, but the two pairs are so closely parallel that their invisibility in the living worms is easily understood. The ventral pair represent the original bladder and the dorsal those formed secondarily (Fig. 17). These dorsal vessels originate from the posterior dorsal regions of the bladder directly after the division of the common excretory duct into two parts (Fig. 17). They extend anteriorad as far as the Laurer's canal where they fuse into a single vessel which continues forward but a very short distance and ends blindly (Fig. 17). Already at this stage there is some indication of the anastomosing of accessory spaces with the main vessels which is found in the adult. This system, as herein described for the metacercaria, is more complex, particularly in the hindbody, than that of any other described *Diplostomulum*. The degree of development of all the organs is unusual in the metacercaria of *A. mustelae*, and the development of the excretory system conforms therewith. The metacercariae of the neascus type as worked out by Hughes show greater development of the excretory system than do most diplostomula, for in the former the four vessels of the hindbody are more or less distinguishable and there are transverse spaces or vessels connecting the several parts, thus making a complex system. The advanced excretory system of the metacercaria of *A. mustelae* in many respects is suggestive of that found in neascus.

The complex excretory system of the adult results from the progressive development of the process of anastomosis begun in the metacercaria. Van Haitma's (1931b: 491-492) detailed account of the excretory system of *Diplostomum flexicaudum* agrees very well with the excretory system of *Alaria mustelae* except in a few points of detail. The system in the hindbody of *A. mustelae* is based on the four vessels found in the metacercaria. The two ventral vessels maintain their identity throughout their length, running along lateral to the descending limb of the uterus, and the commissure formed by their union posterior to the holdfast organ also remains (Fig. 18). The dorsal vessels are almost entirely lost as separate entities in an irregular anastomosing network of peripheral spaces, except at the anterior end of the hindbody where the spaces merge to form a single vessel, which ascends a short distance and unites with the bladder commissure (Fig. 18). At the level of the middle of the ovary the dorsal spaces unite with the ventral peripheral spaces which extend both posteriorad and anteriorad, the latter continuing into the forebody as a series of marginal spaces (Fig. 18).

Van Haitma (1931b) described three lateral vessels on each side and one median vessel in the forebody but did not indicate their connections with the vessels of the hindbody. The system found in the forebody (Fig. 18) is also based on the preëxisting system formed in the metacercaria. The

most median of the lateral vessels which lies near the digestive cecum is the original anterior end of the primary bladder and is connected to the anterior ends of the ventral vessels of the hindbody (Fig. 21). The other lateral vessels were the branches of this bladder in the metacercaria, the posterior branches of which extended into the hindbody. As has been mentioned above the peripheral ventral spaces of the hindbody continue into the forebody as the marginal vessels, the original of which were the posterior branches given off from the anterior primary bladder (Fig. 17), and which are only secondarily connected with the dorsal vessels of the hindbody. The anterior marginal vessels came from the anterior branches of the primary bladder which with the posterior branches form an anastomosing network of marginal vessels (Fig. 18). Certain of the anterior branches by uniting with those of the opposite side form the anterior transverse commissure in the region of the pharynx, the median commissure dorsal to the acetabulum, and a median dorsal vessel which connects them and extends a short distance posteriad dorsal to the holdfast organ. In the adult both median and anterior transverse commissures are represented by an extensive anastomosing network of small vessels, and there are several connections between the median dorsal vessel in the region of the holdfast organ and the lateral vessels on each side (Fig. 18). Van Haitsma (1931b) thought that the median dorsal vessel of the forebody may connect by means of fine tubules with the transverse bladder commissure of the hindbody. That this is not necessary is appreciated when the origin of this median vessel is recalled and the several transverse connections of this vessel with the lateral vessels remembered. The median vessel arises in the metacercaria from the median transverse commissure which sends a branch anteriad and another posteriad, the former uniting with the anterior commissure and the latter ending as a group of blind capillaries containing calcareous corpuscles (Fig. 17). These capillaries in the adult have become the transverse spaces connected to this vessel.

That this is the usual plan of excretory system to be found in strigeids is suggested by Van Haitsma (1931b) who has worked out the excretory systems of four adults representing as many genera, viz. *Crassiphiala bulboglossa*, *Paradiplostomum ptychocheilus*, *Cotylurus flabelliformis*, and *Diplostomum flexicaudum* in all of which it is seen to have a consistent arrangement. To these is now added the genus *Alaria*, having the same general plan.

Krause (1914) named two vessels which he observed running along and lateral to the descending limb of the uterus the "Uterusgefäße." According to several of his figures of cross sections of adult hemistomes these vessels should be interpreted as the ventral vessels or primary bladder which are never lost in the anastomosing network. Other workers (Brandes, 1890; Poirier, 1886; Guberlet, 1922), as pointed out by Van Haitsma, were also

able to distinguish but two longitudinal vessels in the hindbody. Van Haitsma (1931a) stated that this system of vessels is more readily traced in young adults, which would be comparable in most cases to the precociously developed metacercaria of *A. mustelae* in which this system could be followed with ease. It is therefore not surprising that Krause and the other investigators did not get a true picture of this system as they were usually dealing with mature specimens in which the original system had become much obscured by the irregular anastomosing of the accessory spaces with the primary vessels.

A system of vessels more or less comparable to the reserve excretory system of the strigeids is found in the lymph system of monostomes and amphistomes which has been recognized as a definite system since their description by Looss (1902). All investigators have found it to be a closed system without perceptible connections with the excretory channels. The functions of this system, as given by Willey (1930), are the distribution of food and removal of wastes. He pointed out the intimate association of the lymph spaces with the intestinal ceca, providing for the absorption of soluble food into the lymph, and with the organs of greatest activity, supplying the material needed for their metabolism. As an argument for the contention that the lymph system also has an excretory function Willey stressed the extensive distribution of lymph sacs around the excretory vesicle.

That the system of vessels and spaces known as the reserve excretory system of the strigeids has a close connection structurally with the primary excretory system is demonstrated by the origin of this system as stated above, and as shown by the series of developmental stages figured in Pl. 3, and its excretory function assumed on the structural basis. In discussing the function of the excretory system in *Cotylurus flabelliformis* Van Haitsma (1931a) mentioned the limited digestive system as hardly adequate for the distribution of nourishment to all cells of the body and the lack of a circulatory system, and therefore suggested that the reserve excretory system may function in this capacity. The association of the excretory spaces with the food supply is as intimate in the strigeids as is the system of lymph vessels in the amphistomes; and the circulatory function of the reserve excretory system can be based on the same grounds as stated for this functioning of the lymph system in the amphistomes and monostomes. A more complete comparison of the two systems might be made if the complete development of the lymph system were known. It is supposed to originate in the metacercaria and, according to Stunkard (1929), arises "as a series of confluent intercellular spaces and as outgrowths from these primary centers, the lymph system of the amphistomes presents the same developmental features as the vascular system of higher forms." Stunkard, however, did not claim to have followed the development of this system and

most of his work was confined to parasites from the final hosts. We are probably not dealing here with homologous but with analogous structures.

SUMMARY

1. The life history of a new species of *Alaria* has been worked out experimentally from the cercaria to the adult, the first life history of a species of this genus to be completed.

2. The mesocercaria was shown to be an indispensable step in the completion of the life cycle.

3. A series of four hosts were found to be required for this life cycle.

4. The morphology of the respective stages in the life cycle from cercaria to adult have been fully described.

5. The development of the excretory system and of several other organs has been followed from the cercaria to the adult.

BIBLIOGRAPHY

- Ameel, D. J. 1931. More data on the lung fluke, *Paragonimus*, in North America. *Science* (N. S.), 74:493-494.
- Augustine, D. L. and C. Uribe. 1927. *Alaria arisaemoides*, n. sp., a trematode from *Vulpes fulva*. *Parasitol.* 19:236-244.
- Blochmann, F. 1910. Sterben von Aquarienfischen durch Einwanderung von *Cercaria fissicauda* La Val. *Centralbl. f. Bakt. u. Parasitenk.* 1 Abt. Orig. 56:47-49.
- Bosma, N. J. 1931. *Alaria mustelae*, sp. nov., a new trematode requiring four hosts. *Science* (N. S.), 74:521-522.
- Brandes, G. 1890. Die Familie der Holostomiden. *Zool. Jahrb., Abt. f. Syst.* 5:549-604.
- Cort, W. W. 1917. Homologies of the excretory system of the forked-tailed cercariae. *Jour. Parasitol.* 4:49-57.
1918. The excretory system of *Agamodistomum marcianae* (La Rue), the Agamodistomum stage of a forked-tailed cercaria. *Ibid.* 4:130-134.
- Cort, W. W. and S. T. Brooks, 1928. Studies on the holostome cercariae from Douglas Lake, Michigan. *Trans. Am. Micr. Soc.* 47:179-221.
- Dollfus, R. P. 1929. Existe-t-il des cycles évolutifs abrégés chez les trématodes digénétiques? Le cas de *Ratzia parva* (Stossich, 1904). *Ann. de Parasitol.* 7:196-203.
- Dubois, G. 1927. Descriptions de nouveaux trématodes d'oiseaux du genre "*Hemistomum*." *Bul. de la Soc. neuchâteloise d. Sc. nat., nouv. ser.* 1:33-44.
1929. Les cercaires de la région de Neuchâtel. *Ibid., nouv. ser.* 2:1-177.
- Duncker, H. C. J. 1881. Distomeen im Schweinefleisch. *Zeitschr. f. mikr. Fleischschau*, Berlin, 2:23-25.
- Faust, E. C. 1919. The excretory system in Digenea. II. Observations on the excretory system in distome cercariae. *Biol. Bull.* 36:322-339.
- Fraipont, J. 1880. Recherches sur l'appareil excréteur des trématodes et des cestodes. *Arch. de Biol.* 1:415-456.
- Gastaldi, B. 1854. Cenni sopra alcuni nuovi elminti della *Rana esculenta*, con nuove osservazione sul *Codonocephalus mutabilis* Diesing. 14 pp., Torino.
- Giard, A. 1887. Sur la progénèse. Exposé des titres et Travaux scientifiques (1869-1896). *Travaux du Laboratoire de Zoologie maritime de Wimereux*, V.
- Goeze, J. A. E. 1782. Versuch einer Naturgeschichte der Eingeweidewürmer thierischer Körper. XI+471 pp., Blankenburg.
- Guberlet, J. E. 1922. Three new species of Holostomidae. *Jour. Parasitol.* 9:6-14.
- Hall, M. C. and M. Wigdor, 1918. Two new flukes from the dog. *Am. Vet. Med. Assoc. Jour.* 53, n.s. 6:616-626.

- Hughes, R. C. 1927. Studies on the trematode family Strigeidae (Holostomidae). No. VI. A new metacercaria *Neascus ambloplitis*, sp. nov. representing a new larval group. Trans. Am. Micr. Soc. 46:248-267.
- 1928a. Studies on the trematode family Strigeidae (Holostomidae). No. IX. *Neascus van-cleavei* (Agersborg). Trans. Am. Micr. Soc. 47:320-341.
- 1928b. Studies on the trematode family Strigeidae (Holostomidae). No. X. *Neascus bulboglossa* (Van Haitsma). Jour. Parasitol. 15:52-57.
- 1928c. Studies on the trematode family Strigeidae (Holostomidae). No. XII. *Agamodistomum la-ruei* sp. nov. Parasitol. 20:413-420.
- 1929a. Studies on the trematode family Strigeidae (Holostomidae). No. XIV. Two new species of diplostomula. Occasional Papers Mus. Zool., Univ. Mich. No. 202:1-29.
- Krause, R. 1914. Beitrag zur Kenntnis der Hemistominae. Zeitschr. f. wissensch. Zool. 112:93-238.
- La Rue, G. R. 1917. Two new larval trematodes from *Thamnophis marciana* and *Thamnophis eques*. Occasional Papers Mus. Zool., Univ. Mich. No. 35:1-12.
1927. Studies on the trematode family Strigeidae (Holostomidae). No. V. *Proalaria huronensis*, sp. nov. Trans. Amer. Microsc. Soc. 46:26-35.
- La Rue, G. R. and G. H. Barone. 1932. *Alaria oregonensis* from the coyote (Trematoda: Alariidae). Trans. Amer. Microsc. Soc. 51:199-208.
- La Rue, G. R. and E. W. Townsend. 1932. A morphological study of *Alaria nasuae* La Rue and Townsend (Trematoda: Alariidae). Trans. Amer. Microsc. Soc. 51:252-263.
- Leuckart, K. G. F. R. 1889. Die Parasiten des Menschen und die von ihnen herrührenden Krankheiten. 2 Aufl., 1 Bd., 4. Lief., 2 Abt., ix+97-440 pp. Leipzig u. Heidelberg.
- Linstow, O. F. B. von. 1875. Beobachtungen an neuen und bekannten Helminthen. Arch. f. Naturgesch. 41:183-207.
1876. Helminthologische Beobachtungen. Ibid. 42:1-18.
1877. Enthelminthologica. Ibid. 43:173-198.
- Looss, A. 1902. Ueber neue und bekannte Trematoden aus Seeschildkröten. Zool. Jahrb., Abt. f. Syst. 16:411-894.
- Lutz, A. 1921. Zur Kenntnis des Entwicklungszyklus der Holostomiden. Centralbl. f. Bakt. u. Parasitenk. 1 Abt. Orig. 86:124-129.
1929. Nova contribuição para o conhecimento do cyclo evolutivo das Holostomídeas ou Strigeídeas. Mem. Inst. Oswaldo Cruz, Suppl., No. 8, 128-130.
- Mathias, P. 1925. Recherches expérimentales sur le cycle évolutif de quelques trématodes. Bull. Biol. France et Belg. 59: 1-123.
- Miller, H. M. 1926. Comparative studies of furcocercous cercariae. Ill. Biol. Monogr. 10(3): 1-112.
1927. Furcocercous larval trematodes from San Juan Island, Washington. Parasitol. 19:61-83.
- Molin, R. 1858. Prospectus helminthum, quae in prodromo faunae helminthologicae Venetiae continentur. Sitzungsber. d. k. Akad. d. Wiss. Wien, math.-nat. Kl. 30:127-158.
- Poirier, J. 1886. Sur les Diplostomidae. Arch. de Zool. expér. et gén., Paris 2 s. 4:327-346.
- Railliet, A. 1919. Nouveaux trématodes du chien, par Hall et Wigdor. Rec. d. ———— ve't. 95: 229-232.
- Ruszkowski, J. 1922. Die Postembryonale Entwicklung von *Hemistomum alatum* Dies. auf Grund experimenteller Untersuchungen. Bull. d l'Acad. Polon. Sci. et Lettres, Cl. d. Sci. Math. e. Nat., ser. B, Sci. Nat. 237-250.
- Schrank, F. v. P. 1788. Verzeichniss der bisher hinlänglich bekannten Eingeweidewürmer, nebst einer Abhandlung über ihre Anverwandtschaften. v+116 pp. 1 table, 12°, München (Kgl. Svenska Vetensk. Akad., Stockholm).
- Sewell, R. B. S. 1922. Cercariae Indiceae. Indian Jour. of Med. Res. 10 (Suppl.), 370 pp.
- Stiles, C. W. and A. Hassall. 1900. A muscle fluke (*Agamodistomum* sp.) in American swine (pp. 559-560, fig. 23). Notes on Parasites 50-52. U. S. Bur. An. Ind., 16th. Ann. Rep. 558-611.

- Stunkard, H. W. 1929. The parasitic worms collected by the American Museum of Natural History Expedition to the Belgian Congo, 1909-1914. Part I Trematoda. Bull. Am. Mus. Nat. Hist. 58:233-289.
- Szidat, L. 1924a. Beiträge zur Entwicklungsgeschichte der Holostomiden I. Zool. Anz. 58: 299-314.
- 1924b. Beiträge zur Entwicklungsgeschichte der Holostomiden II. Entwicklung der Cercaria C. Ibid. 61:249-266.
- 1929a. Beiträge zur Kenntnis der Gattung *Strigea* (Abbild.) I. Allgemeiner Teil: Untersuchungen über die Morphologie, Physiologie, und Entwicklungsgeschichte der Holostomiden nebst Bemerkungen über die Metamorphose der Trematoden und die Phylogenie derselben. Zeit f. Parasitenk. (Abt. F. der Zeit. f. wissensch. Biol.), 1:612-687.
- 1929b. Beiträge zur Kenntnis der Gattung *Strigea* (Abbild.) II. Spezieller Teil: Revision der Gattung *Strigea* nebst Beschreibung einer Anzahl neuer Gattungen und Arten. Ibid. 1:688-764.
- Van Haitsma, J. P. 1930. Studies on the trematode family Strigeidae (Holostomidae) No. XXI. Life-cycle and description of the cercaria of *Cotylurus michiganensis* (La Rue). Jour Parasitol. 16:224-230.
- 1931a. Studies on the trematode family Strigeidae (Holostomidae) No. XXII. *Cotylurus fiabelliformis* (Faust) and its life-history. Mich. Acad. Sci., Arts and Letters, 13:447-482.
- 1931b. Studies on the trematode family Strigeidae (Holostomidae) No. XXIII. *Diplostomum flexicaudum* (Cort and Brooks) and stages in its life-history. Ibid. 13:483-516.
- Wiley, C. H. 1930. Studies on the lymph system of digenetic trematodes. Jour. Morph. 50: 1-37.

EXPLANATION OF PLATES

Abbreviations

<i>ab</i> —anterior limb of bladder	<i>ovd</i> —oviduct
<i>act</i> —anterior collecting tube	<i>pct</i> —posterior collecting tube
<i>bc</i> —bursa copulatrix	<i>pg</i> —penetration glands
<i>bl</i> —excretory bladder	<i>sr</i> —seminal reservoir
<i>cvd</i> —common vitelline duct	<i>sv</i> —seminal vesicle
<i>dej</i> —ductus ejaculatorius	<i>t₁</i> —testis, anterior
<i>dex</i> —dorsal excretory vessels	<i>t₂</i> —testis, posterior
<i>exp</i> —excretory pore	<i>ues</i> —unpigmented eyespot
<i>ga</i> —genital atrium	<i>uta</i> —uterus, ascending limb
<i>gp</i> —genital primordium	<i>utd</i> —uterus, descending limb
<i>int</i> —intestine	<i>vd</i> —vas deferens
<i>lc</i> —Laurer's canal	<i>ve₁</i> —vas efferens of <i>t₁</i>
<i>lsc</i> —lateral sucking cups	<i>ve₂</i> —vas efferens of <i>t₂</i>
<i>mg</i> —Mehlis' gland	<i>vps</i> —ventral peripheral excretory spaces
<i>oot</i> —oötype	<i>vr</i> —vitelline reservoir
<i>ov</i> —ovary	

EXPLANATION OF PLATE VIII

- FIG. 1. Camera lucida outline drawings of a series of 5 adults taken from one dog, showing various contraction states of body and holdfast organ.
- FIG. 2. Mature metacercaria.
- FIG. 3. Reconstruction of the hindbody of a mature metacercaria showing reproductive system in sagittal view.
- FIG. 4. Mesocercaria.
- FIG. 5. Cercaria, semi-diagrammatic.

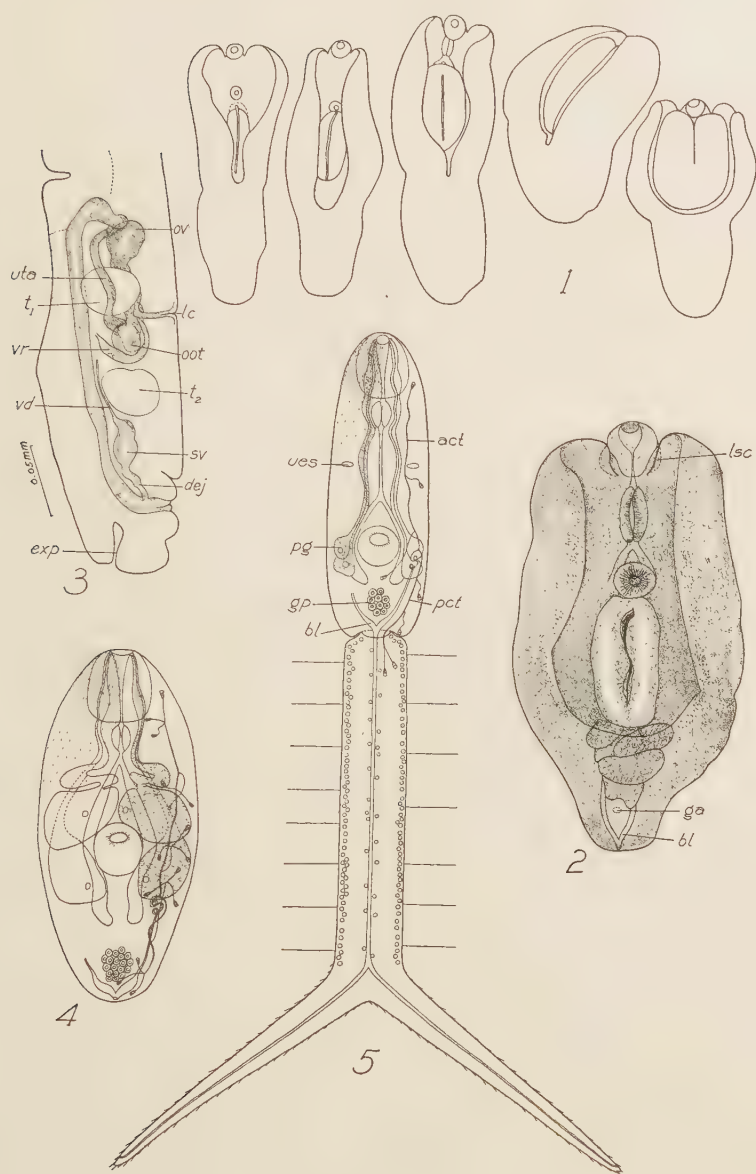


PLATE VIII

EXPLANATION OF PLATE IX

- FIG. 6. Reconstruction of transverse sections showing relation of vasa efferentia and vas deferens to the seminal reservoir, the latter being anterior to t_1 . Anterior aspect.
- FIG. 7. Reconstruction of cross sections, somewhat posterior to that of fig. 6, showing origin of oviduct and Laurer's canal, union of common vitelline duct and oviduct, and extent of Mehlis' gland, marked by dotted line. Anterior aspect.
- FIG. 8. Reconstruction of cross sections at a level between the testes, showing vitelline reservoir, Mehlis' gland, oötype, and loop made by uterus between the testes before it ascends. Anterior aspect.
- FIG. 9. Cross section through posterior testis, showing relation of posterior vas efferens, vas deferens and uterus. Anterior aspect.
- FIG. 10. Reconstruction of male reproductive system as seen from left side in sagittal view.
- FIG. 11. Reconstruction of female reproductive system as seen from left side in sagittal view.

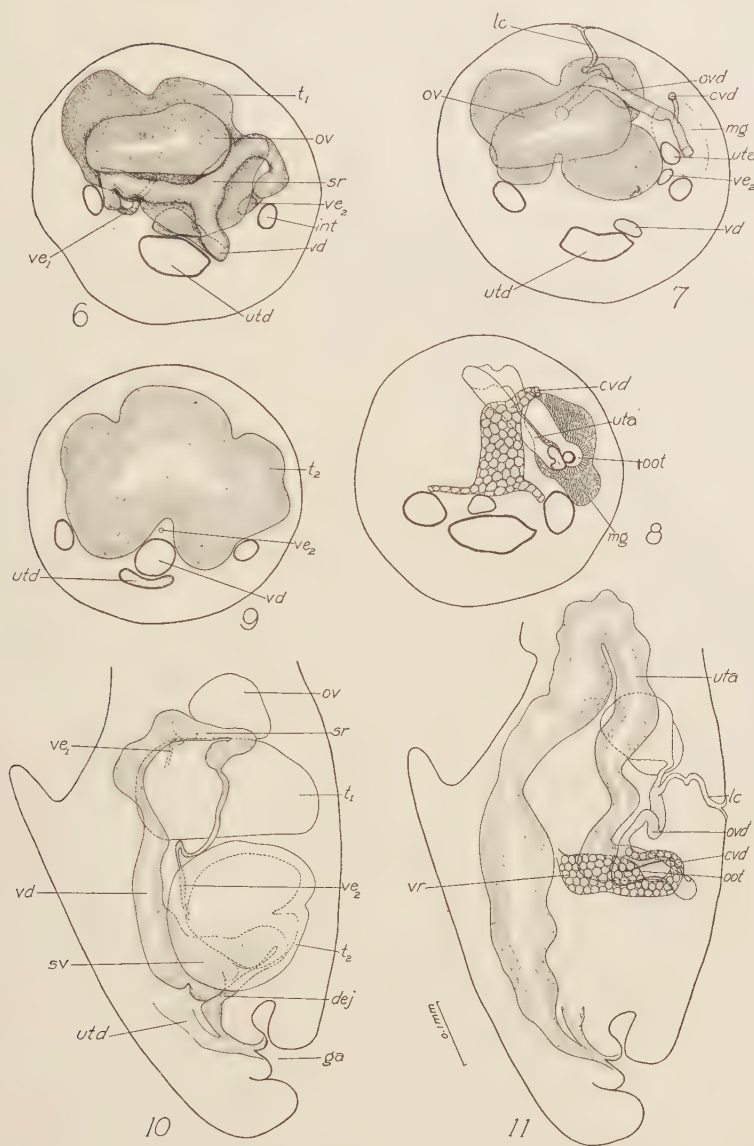


PLATE IX

EXPLANATION OF PLATE X

FIG. 12. Cercaria.

FIG. 13. Mesocercaria.

FIG. 14. Early metacercaria showing mesiad bending of bladder, the beginning of the formation of the bladder commissure. The many flame cells attached to collecting tubules not shown.

FIGS. 15-16. Developing metacercariae showing continuation of growth of the bladder limbs toward formation of the bladder commissure, and the beginning of the reserve excretory system.

FIG. 17. Mature metacercaria with complete bladder commissure, dorsal vessels in hindbody, shown in broken lines, branching of reserve system complete.

FIG. 18. Adult excretory system, from ventral aspect, consisting of an anastomosing network. Ventral bladder vessels shown as tube; all white spaces of the hindbody represent ventral peripheral spaces with trabeculae of tissue between them, shown as shaded areas; origin and terminus of dorsal vessels shown by dotted lines. Connections of ventral peripheral spaces of hindbody to marginal vessels of forebody should be noted. The collecting tubules figured in earlier stages could not be traced in adult, although they must be present, *a*, *b*, *c* and *d* levels at which figs. 20, 21, 22 and 23 were taken. All sexual and digestive organs omitted.

FIG. 19. Dorsal aspect of the excretory system of the hindbody of the adult.

FIG. 20. Cross section of forebody of adult at level marked *a* in fig. 18. The union of the dorsal vessel to the anterior portion of the bladder commissure shown. Intestine shown as heavy walled in this and following figures.

FIG. 21. Cross section of hindbody of adult at level marked *b* in fig. 18. Ovary shaded. Union of original anterior limb of bladder extending into the forebody, with the bladder of hindbody labelled *ab*.

FIG. 22. Cross section through testis at level *c* in fig. 18, showing ventral bladder vessels, dorsal and ventral peripheral spaces.

FIG. 23. Cross section at level *d* in fig. 18, showing portion of genital atrium, and the dorsal excretory vessels shortly after their origin from the ventral vessels.

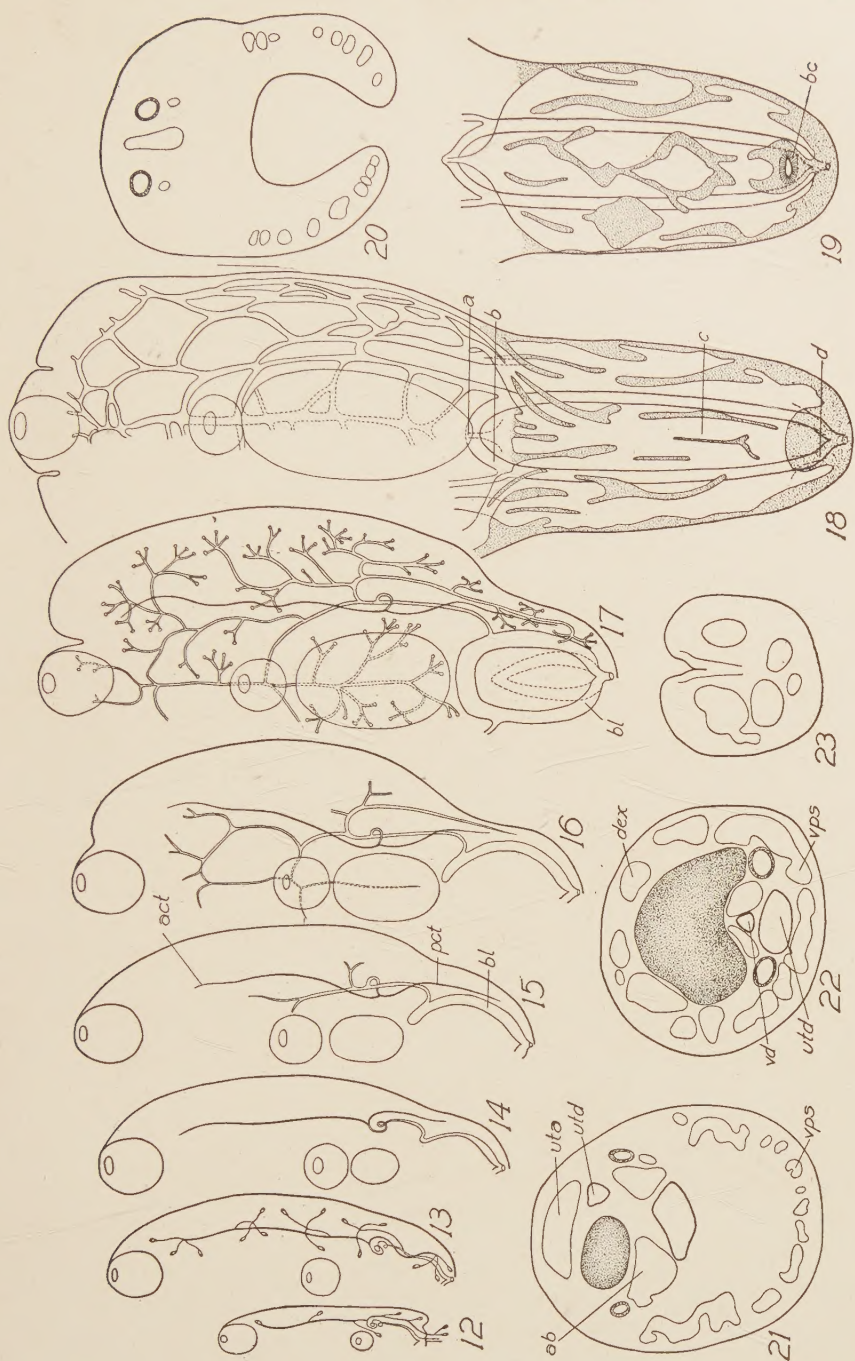


PLATE X



